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Review

Lipid rafts and their roles in T-cell activation

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Abstract

Lipid rafts are defined as detergent-resistant membrane microdomains of specific lipid and protein composition. They are involved in many aspects of cell biology, including T-cell activation and immunoreceptor signaling. This review discusses current controversies around lipid rafts and summarizes recent developments in the area.
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1. Introduction

It has been known for a long time that T cells can be activated by antibody-mediated cross-linking of surface glycoproteins such as Thy-1 or Ly-6. This finding became very surprising when it was found that these molecules are anchored in the plasma membrane by means of a glycolipid moiety, glycosylphosphatidylinositol (GPI), covalently attached to their C-termini. It turned out that many, if not all, GPI-anchored proteins can elicit in various types of leukocytes, upon suitable cross-linking, activating signals mimicking in their effects, at least in some aspects, activation induced by immunoreceptors, such as T-cell receptor (TCR), B-cell receptor (BCR) or some Fc-receptors. Moreover, similar activation could be achieved by cross-linking of certain glycolipids. The signaling capacity of the GPI-anchored glycoproteins was clearly dependent on the GPI-anchor, as transmembrane versions did not signal (for review on these aspects see [1]). The question was “how can molecules devoid of any transmembrane and cytoplasmic domains trigger the intracellular signaling cascades, dependent on cytoplasmic molecules such as Src- and Syk-family kinases and phospholipase C?” A possible explanation came after the discovery of so-called lipid rafts (also called GEMs)—membrane microdomains enriched in glycosphingolipids, cholesterol and lipid-modified proteins, including the GPI-anchored ones. Importantly, these structures are enriched in double-acylated (myristoylated, palmitoylated) Src-family kinases and some other signaling molecules (see below). Thus, it is conceivable that effective cross-linking of extracellular components of the rafts (glycolipids, GPI-proteins) may cause redistribution (clustering) of the raft-associated cytoplasmic Src-family kinases, which start to transphosphorylate each other and subsequently phosphorylate other substrates normally involved in immunoreceptor signaling. However, lipid rafts turned out to be much more important—it was soon found that they are critically involved in early phases of immunoreceptor signaling. It should be noted that this raft-based model of immunoreceptor signaling, described in some detail below, is not mutually exclusive with a more traditional model postulating that at least a minor fraction of the immunoreceptors is pre-associated with the Src kinases and that such complexes act in a classical receptor-kinase manner, more or less independently of the rafts.

Numerous recent reviews on various aspects of membrane rafts and their involvement in immunoreceptor signaling exist [1–6]. Due to space limitations, in this short review, I will not give references for all the facts mentioned here; the readers are mainly referred to the above more detailed reviews.

2. Basic properties of lipid rafts

Due to their unique lipid composition, lipid rafts are relatively resistant to solubilization at low-temperature by some common detergents, such as Triton X-100, Brij-series, NP-40 or CHAPS. This property makes it possible to separate them easily from the other more fully solubilized mem-

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brane components by density gradient ultracentrifugation. These membrane structures are apparently held together mainly by hydrophobic interactions between saturated fatty acid residues of their main lipid constituents, sphingomyelin and glycosphingolipids (such as GM3 in T lymphocytes) and further stabilized by intercalated cholesterol molecules. While it is clear that the outer leaflet of the rafts is selectively enriched in glycosphingolipids, it is less clear whether both leaflets of the raft's bilayer have similar basic lipid composition (with respect to lipids with saturated fatty acids and cholesterol). Another lipid important for maintaining (and aggregation) of the rafts is ceramide, a product of cleavage of sphingolipids by acidic sphingomyelinase. Interestingly, lipid rafts also exist in cells deficient in expression of GPI-anchored proteins [7] or deficient in glycosphingolipid synthesis [8], indicating that these components are not crucial for their integrity. However, lipid rafts are at least partially dissociated by cholesterol depletion [9] or biosynthetic replacement of saturated fatty acid residues in their sphingolipids by unsaturated ones [10].

As stated above, characteristic protein components of lipid rafts are extracellularly oriented, GPI-anchored proteins (in T cells e.g. CD48, CD52, CD55, CD59, CD90 (Thy-1), CD108, CD230 (prion protein) or Ly-6). Most transmembrane proteins are excluded, except for several palmitoylated ones, such as the co-receptors CD4 and CD8β, pre-TCR (in early thymocytes), adhesion receptor CD44, proteolipid MAL, influenza virus hemagglutinin, several members of the TNF-receptor family and transmembrane adaptor proteins LAT, PAG, NTAL and LIME (see below). Palmitoylation-deficient mutants of several of the raft resident proteins such as Src-family PTKs, CD8β, pre-TCR or LAT [11–15] were shown to be excluded from the rafts. Compared to the bulk membrane, membrane rafts appear to possess a thicker membrane, which corresponds to their enrichment in lipids possessing long, saturated (extended) fatty acids.

3. Problematic aspects of the rafts

The most popular current idea about lipid rafts is that they are tiny "islets" that cannot be observed easily on intact cell surface by existing microscopic techniques. Actually, a dot-like distribution of GPI-anchored proteins or glycolipids apparently corresponding to lipid rafts can be observed after visualization by means of fluorescently labeled antibodies or cholera toxin. However, this is probably mainly due to aggregation induced by these multivalent reagents (cholera toxin has five binding sites for the GM1 glycolipid). Distribution of GPI-anchored intrinsically fluorescent proteins or lipids is essentially homogeneous [16], indicating probably that the size of unperturbed rafts is under the limit of optical microscopy.

Therefore, most of the available data are based on analysis of the preparations obtained by density gradient ultracentrifugation of low-temperature detergent lysates. This is, of course, a problem: it is not clear to what extent the composition and properties of "native" rafts in the unperturbed membrane correspond to those obtained following detergent extraction at low-temperature. It is entirely plausible that some components present in the lipid rafts in vivo are lost by detergent extraction, while the presence of a detergent at low-temperature may induce artificial associations and even lipid phase transitions. The problem is further complicated by the fact that the composition of the isolated rafts is markedly dependent on the conditions used for their preparation, especially the nature and concentration of the detergent used. Milder detergents (Brij-98, Brij-58, Lubrol) produce higher yields of buoyant rafts containing a majority of total cellular GPI-anchored proteins, transmembrane adaptors LAT or PAG/Cbp, and substantial fractions of Src-family kinases but also small amounts of several "atypical" transmembrane proteins. The use of more stringent 1% detergents Triton X-100 or NP40 typically produces much less buoyant fractions containing only less than 50% of the typical raft molecules (GPI-proteins, transmembrane adaptors, Src kinases, CD4) and only a few non-palmitoylated transmembrane proteins. The simplest explanation is that the very mild detergents best preserve the native structures and that the "atypical" components (CD45, etc.) are indeed attached weakly to native rafts (these hypothetical native structures might consist of a core resistant even to the more stringent detergents and a periphery more sensitive to Triton X-100 but resistant to Brij-58). Another possibility is that there are several more or less distinct types of rafts, differing in sensitivity to different detergents. Various other interpretations are possible; this issue was recently excellently discussed by Pike [17].

Because of these problems, the very existence of lipid rafts in vivo has been repeatedly put in doubt. However, several recent experimental approaches do support, in my opinion quite strongly, their reality in living cells (see [1] for references).

- Chemical and biophysical methods, such as chemical cross-linking, single particle tracking, fluorescence energy transfer, laser trap or single dye tracking, indicated that the lipid and protein components are confined within cholesterol-dependent membrane microdomains sized 40 nm to more than 1 μm depending on the cell type and the method used.
- Lipid rafts can be isolated even at 37 °C if using detergent Brij-98, which excludes the objection that low-temperature solubilization induces lipid phase transitions, artificially creating the detergent-resistant protein–lipid complexes. Actually, membrane fragments very similar to lipid rafts can be obtained by membrane disintegration in the absence of any detergent (albeit at low-temperature).
- Spontaneous formation at physiological conditions of entities remarkably resembling lipid rafts could be demonstrated in model membranes and liposomes composed of well-defined lipid mixtures.

An important point relevant to discussions on the existence and nature of lipid rafts in vivo is what percentage of the

cell surface is actually covered by the rafts. In polarized epithelial cell, most of the apical surface appears to be composed of the rafts. Although earlier estimates for other cell types, such as leukocytes, were in the range 5–10% (based on recoveries of lipid and protein components from Triton X-100-insoluble rafts), more recent consensus values are around 50%. The situation may then resemble a lake covered so densely by mutually contacting rafts (which even readily lose and exchange their logs) that a distant view cannot distinguish the individual rafts. Speaking less metaphorically, the cell surface may be a complex and dynamic mosaic of various types of microdomains in which a specific type (as characterized by a particular marker such as a GPI-anchored protein) may be almost impossible to be distinguished by even a very high-power microscopic technique. Furthermore, it is a common observation that most of the "typical raft markers" are actually also markedly present in the detergent-sensitive fractions of the membranes, indicating that under physiological conditions, separate raft- and non-raft fractions of these molecules exist (differing either by subtle structural modifications, such as oligomerization, or distributed simply due to a dynamic physico-chemically driven exchange between raft and non-raft compartments). Thus, attempts to visualize the rafts as distinct dot-like structures may be rather futile.

Another relatively underestimated problem is potential heterogeneity of rafts in a given cell type: in contrast to the prevailing view that essentially a single type of rafts exists at a given cell surface, there could be rather several, or even many types, each enriched in specific molecules only (e.g. one Lck-rich, another LAT-rich, etc.). Recent data indicate that this is indeed the case. For example, two clearly distinct types of rafts were demonstrated in motile T cells, one of them originating from the leading edge and the other from the uropod [18]; these were characterized by the specific enrichment by two major glycolipids, GM1 and GM3, respectively. Separate microdomains enriched in Lck or LAT, respectively, and differentially sensitive to cholesterol depletion were demonstrated in the membranes of T-cell lymphoblasts [19]. Furthermore, as mentioned above, differences in the composition and properties of lipid rafts prepared by means of different detergents may also suggest the existence of several types of native rafts.

It is therefore very likely that the standard methods of raft isolation produce a mixture of different rafts difficult to further separate, and this may contribute to some confusion. Improved techniques addressing this problem, e.g. the use of shallow density gradients or methods combining separation according to density and size, should help to clarify this issue.

Thus far, only buoyant rafts have been studied. It is, however, entirely possible that "heavy rafts" with a higher protein/lipid ratio exist and are hidden in the bottom fraction of the gradient mixed with the completely solubilized proteins. A simple approach to discover and study such hypothetical atypical membrane rafts might be based on the use of optimally engineered gradients and/or on separation of the heavy rafts from the complex mixture in the dense fractions of the gradient by sizing methods. Cholesterol-independent, temperature-resistant raft-like microdomains different from the "classical rafts" were described containing several tetraspanin proteins and phosphatidylinositol 4-kinase [20].

A widely used approach to demonstrate involvement of lipid rafts in cellular functions has been based on removal of cholesterol, mainly by extraction with methyl-β-cyclodextrin (MβCD), complexing it with other agents (filipin, nystatin) or modifying it with cholesterol oxidase. Thus, if some cellular function is affected by such treatments, it is taken as evidence for raft involvement. However, these treatments are not specific for cholesterol present in rafts and affect also the fraction of this membrane lipid present in the non-raft membrane.

Another useful treatment targeting rafts is based on biosynthetic replacement of a fraction of saturated fatty acids in the membrane lipids by polyunsaturated ones [10], resulting in partial displacement of Src-family kinases and LAT from the detergent-resistant rafts. Interestingly, GPI-anchored proteins and ganglioside GM1, both residing in the outer membrane leaflet, remained in the buoyant rafts, indicating that this particular treatment probably selectively modifies the cytoplasmic layer of membrane rafts. Lipid rafts can also be targeted in vivo by exposure of cells to short-chain ceramides [21].

Although there is presently no definitive consensus, it seems likely that "elementary rafts" are quite small (diameter < 10 nm) and contain very few (perhaps even single, and some, none at all) protein molecules surrounded by a "shell" of about 100 of the specific lipid molecules. Lipid composition of these lipid shells around various membrane proteins is probably dictated by properties of the transmembrane domains, other membrane-interacting areas of the proteins and/or their lipid modifications. Such elementary microdomains probably may coalesce with some specificity into larger patches, either after engagement of their protein or glycolipid components with their natural ligands, after artificial cross-linking by antibodies or after exposure to some types of mild detergents. A major function of lipid rafts may be to accumulate signaling molecules and keep them in resting state separated from relevant receptors.

A really strong indication of the real existence of functionally important lipid rafts under physiological conditions, whatever their size or other properties are, is the fact that palmitoylation-deficient mutants of several signaling proteins are largely functionally impaired (although they are still anchored in the detergent-soluble non-raft membrane) [11–15]. In my opinion, some of the studies apparently questioning the existence of lipid rafts in vivo are based on false assumptions. For example, some of these studies assume that the GPI-anchored proteins must be clustered in the rafts; lack of such clustering as indicated by absence of efficient fluorescence resonance energy transfer (FRET) between the fluorescently tagged molecules is taken as evidence against the rafts. However, it is entirely plausible that distances between individual proteins within a single raft are too large to allow

for efficient FRET. Also, the frequent intuitive assumption that the diffusion rate of proteins should be lower within the rafts, or that there should be sharp boundaries between the raft and non-raft membrane markedly restricting the movement of the raft components, may not be substantiated.

4. Involvement of lipid rafts in TCR signaling

Lipid rafts became really interesting for immunologists when it was found that they may play essential roles in signaling initiated through TCR, BCR, several Fc-receptors and possibly other receptors as well. An intriguing point was that the T-cell activation elicited by cross-linking of GPI-anchored proteins is dependent on the expression of the TCR complex, namely the ζ chain. This suggested an intimate link between the lipid rafts and immunoreceptor (such as TCR) signaling. Indeed, biochemical studies in mast cells and T and B lymphocytes revealed that cross-linking of the respective immunoreceptors (FcϵRI, TCR, BCR) results in an association of the clustered receptors with the detergent-resistant lipid rafts. This can be conveniently detected by density gradient ultracentrifugation of cold detergent-solubilized membranes from the activated cells and analysis of the buoyant fractions. Thus, according to a currently popular model, these receptors, upon cross-linking by their natural ligands or by antibodies, aggregate and then merge with lipid rafts. As a result, the tyrosine-based activation motifs (ITAM) in cytoplasmic tails of immunoreceptor complexes (CD3, ζ chain, Igα/β, Fc-receptor γ chain) become exposed to Src-family kinases richly present in the rafts. Phosphorylated ITAMs then serve as docking sites for tandem SH2 domains of Syk-family kinases (ZAP70 or Syk). Activated ZAP70 in T cells phosphorylates another important lipid raft component—the transmembrane adaptor protein LAT.

Phsophorylated LAT binds several cytoplasmic SH2-containing molecules, such as adaptors Grb2 and Gads (and through them, indirectly, other critical signaling molecules such as Sos1, SLP-76, Vav and Itk), as well as a key enzyme, phospholipase Cγ1, which subsequently cleaves yet another raft constituent, phosphatidylinositol 4,5 bisphosphate (PIP_2). One of the products, diacylglycerol, remains in the lipid rafts and attracts protein kinase C isoenzymes. Furthermore, PIP_2 itself and especially its derivative, PIP_3, arising from the activity of PI3 kinase, also serve as docking sites for important signaling molecules carrying PH domains, e.g. Tec-family kinases. Yet another important aspect is that the TCR co-receptors CD4 and CD8 are also palmitoylated proteins associated with lipid rafts. Therefore, their co-engagement with TCR after contact of the T-cell with the antigen-presenting cell (APC), may substantially contribute to co-aggregation of the receptor complex with lipid rafts. An alternative possibility is that TCR may actually be pre-associated with membrane rafts [22], and its ligation just somehow reorganizes this assembly to allow for optimal exposure of the CD3 and ζ chains to the Src-family kinases. This simple model also nicely explains the puzzling observations that cross-linking of GPI-anchored proteins such as Thy-1 results in activation similar to that elicited by TCR cross-linking: apparently, aggregation of lipid rafts also causes enhanced interactions with TCR complexes (due to a non-specific entrapment) followed by tyrosine phosphorylation of their ITAMs. Interestingly, constitutive association of the pre-TCR with lipid rafts is apparently important for generation of pre-thymocyte survival signals [13]. Further strong support for the role of lipid rafts in TCR signaling comes from the previously mentioned observations that palmitoylation-deficient (and raft excluded) mutants of Lck and LAT are functionally defective. In addition, artificial targeting of other cytoplasmic molecules, such as SHP-1 [23], CD45 [24] or PLCγ [25], to membrane rafts has marked functional effects on TCR-induced signaling. Critical sites essential for effective raft association were identified within the TCR complex (a connecting peptide in the TCR α chain and the CD3δ chain). Mutations in these components severely interfere with TCR signaling [26,27]. The basic mechanism of directing association of the engaged TCR complex with lipid rafts is most likely based on co-engagement of the raft resident CD4/CD8 co-receptors mediated by these critical sites in the TCR/CD3 complex.

However, the roles of lipid rafts in TCR signaling remain controversial. Some studies indicate that in T cells stimulated with antigen-pulsed APCs rather than with anti-CD3 antibodies, the initial phases of TCR signaling may be largely independent of lipid rafts [28]. The TCR–raft interactions may be more important in later phases of the signaling or in the process of costimulation through CD28. Indeed, rapid ζ chain phosphorylation is almost normal in T cells expressing defective TCR complexes unable to associate with rafts, while severe defects are observed in distal parts of the TCR signaling pathways [26,27]. Furthermore, if non-specific effects of cholesterol depletion are properly taken into account, there may essentially be no inhibition of ζ chain phosphorylation and cytoplasmic Ca^{2+} elevation after raft dissociation by MβCD treatment. In contrast, signaling through GPI-anchored proteins absolutely requires raft integrity [28]. However, one intriguing hypothetical possibility is that early phases of TCR signaling are dependent on an atypical kind of MβCD-resistant raft.

In naive T cells, a major part of lipid rafts attracted to the clustered TCR seems to come from an unidentified intracellular compartment. In contrast, memory T cells apparently contain more lipid rafts on their surface, and this contributes to their well-known higher sensitivity to antigen stimulation and lack of need for costimulation [29].

An interesting (yet largely speculative) two-step model suggests that two types of rafts may be involved in TCR signaling: those present on the T-cell surface and possibly pre-associated with TCR may be involved in the initial phases, while another type, stored intracellularly, translocates later to the aggregated TCR from intracellular compartments and is involved in costimulation and signal amplification [30].

The TCR–raft interaction seems to be negatively regulated by a cytoplasmic adaptor protein, Cbl-b. Elimination of

this molecule by gene knockout results in enhanced TCR-induced raft aggregation accompanied by sustained tyrosine phosphorylation, causing hyperproliferation of the T cells and spontaneous autoimmunity in the Cbl-b knockout mice [31].

An incompletely resolved issue is involvement of lipid rafts in the "immunological synapse" between the T-cell and APC. Although rafts would be expected to accumulate in the central region of the mature synapse rich in TCR and costimulatory molecules, in a recent study, the only raft marker accumulating in this area was the transmembrane adaptor protein LAT but not several other typical raft markers [32]. Thus, only one type of raft (LAT-enriched) may be specifically recruited to the synapse. However, the actual role of immunological synapse in TCR signaling remains generally controversial.

The interactions of lipid rafts with clustered TCR and immunological synapse may be regulated through their connections to actin cytoskeleton mediated by PAG/Cbp, a raft resident transmembrane adaptor protein (see below). The C-terminal motif of PAG/Cbp binds to the PDZ domain of another cytoplasmic adaptor protein, EBP50 (also known as NHERF), a linker to the ezrin/radixin/moesin proteins bound to fibrillar actin. PAG/Cbp was reported to dissociate from EBP50 following TCR engagement, thereby allowing lipid rafts to move more freely [33].

Various T-cell subsets and various TCR signaling modes may differ with respect to the interactions between TCR and lipid rafts. Thus, TCR signaling in activated Th1 but not Th2 cells is raft dependent [34]. TCRs of immature ($CD4^+CD8^+$) thymocytes and anergic T cells apparently do not use lipid rafts, and the final outcome of this "incomplete" type of signaling results in apoptosis [35,36]. Apoptosis also results if TCRs of mature T cells are prevented from effective association with lipid rafts [37]. As mentioned above, effector and memory T cells have more surface rafts compared to naïve T cells, and this may be the reason for much easier, costimulation-independent triggering of their activation via TCRs. Furthermore, lipid rafts of resting memory T cells are much richer in signaling molecules (including CD45) than are naive cells [38]. Thus, enhanced memory cell responses may be due to this pre-organization of important signaling molecules. Changes in lipid and protein composition of lipid rafts may be also involved in the well-known functional impairment of T cells in aged individuals [39].

5. Roles of lipid rafts in other aspects of T-cell biology

In addition to their direct involvement in TCR (and CD4/CD8 co-receptor) signaling, lipid rafts also play significant roles in several other aspects of T-cell biology.

- A controversial issue has been whether raft-associated Src-family kinases are in an active or rather inactive state. Although the major positive regulator of these enzymes, membrane phosphatase CD45, is largely excluded from lipid rafts, a minor fraction of CD45 apparently is present in the rafts and activates the Src kinases by dephosphorylation of the regulatory C-terminal phosphotyrosine [40]. Importantly, a fraction of the negative regulator of Src-family kinase activity, the PTK Csk, is also found in the rafts, due to its association with the phosphorylated transmembrane adaptor proteins PAG (also called Cbp) [41,42] and LIME [43]. Cross-linking of TCR on resting αβT cells results in rapid transient dephosphorylation of PAG/Cbp and release of Csk [41], leading to increased Src PTK activity needed for early phases of TCR signaling. This regulatory system is even more complex: protein kinase A type I, which is also directed to the rafts of activated T cells (presumably due to its association with a thus far unidentified raft resident scaffolding protein), phosphorylates and thereby activates the raft-associated Csk and thus contributes to the inhibition of Src-family PTKs [44]. Another raft-associated transmembrane adaptor, LIME, becomes tyrosine phosphorylated and Csk associated after cross-linking of the CD4 or CD8 co-receptors [43]; this may contribute to the poorly characterized inhibitory co-receptor signaling. Thus, the appropriate level of activity of the Src kinases present in lipid rafts is maintained by opposing activities of Csk (anchored to adaptor proteins) and CD45.
- Critical costimulatory receptor of T cells, CD28, is a non-raft molecule in the resting state, but following cross-linking, it is re-distributed to the rafts, and this may be important for its co-stimulatory activity [45]; among the mechanisms involved is interaction of a cytoplasmic CD28 motif with SH3 domain of the raft-associated Lck, which markedly increases its kinase activity [46]. Interestingly, the major negative regulator of T-cell activation and CD28 competitor, CTLA-4 (CD152), is constitutively associated with membrane rafts of activated T cells and apparently inhibits redistribution of intracellular rafts to the surface of activated T cells [47].
- Several other signaling molecules are associated with lipid rafts, and following TCR cross-linking, thus associate with the clustered receptors. One of them is PKCθ, which after tyrosine phosphorylation, physically associates with Lck and translocates to lipid rafts associated with immunological synapse [48]. Another constitutive raft resident molecule is the critical regulator of TCR-induced NF-κB activation, scaffolding protein CARMA1 [31].
- Lipid rafts are involved in the functioning of some cytokine receptors, such as IL-2 and IFNγ receptors [49,50].
- Both types of HIV receptors (CD4 and the chemokine receptors) reside in membrane rafts, and the particular lipid environment is necessary for HIV infectivity as well as for virus particle budding [51]. One of the critical pathogenic components of HIV, the myristoylated protein Nef, is targeted to lipid rafts, and this feature seems to be important in its pathogenic ability to prime T cells for activation [52]. Another raft-seeking pathogen is measles virus, which directly interacts with the rafts on human primary T cells, alters recruitment and segregation of membrane proximal

signaling components and thereby suppresses T-cell activation [53].

- Interestingly, a functionally important fraction of MHC class II proteins is found in lipid rafts on the APC surface [54]. This specific lipid environment may help to maintain clusters of these antigen-presenting molecules in an arrangement optimal for recognition by TCR. Thus, lipid rafts are important on both sides of the T-cell-APC contact site.
- Activation of apoptosis-inducing receptors of the TNF-receptor family and death-inducing signaling complex formation occur in lipid rafts. TCR restimulation of activated human $CD4^+$ T cells results in translocation of Fas (CD95) into lipid raft microdomains before binding FasL, rendering these cells sensitive to apoptosis after exposure to CD95 cross-linking antibody or FasL [55]. Interestingly (and somewhat paradoxically), depletion of membrane cholesterol causes ligand-independent spontaneous activation of Fas and apoptosis [56]. It has been shown that TNF-R1 is localized to both lipid raft and non-raft regions of the plasma membrane and that each compartment is capable of initiating different signaling responses [57]. Thus, segregation of TNF-R1 to raft and non-raft regions of the plasma membrane evidently contributes to the diversity of signaling responses initiated by TNF-R1.
- Alterations in lipid raft composition and dynamics apparently contribute to abnormal T-cell responses in systemic lupus erythematosus (SLE). SLE T cells possess higher amounts of lipid rafts and, unlike normal T cells, SLE T-cell lipid rafts include FcRγ chain and activated Syk kinase [58]. Furthermore, localization into lipid rafts of CD45 tyrosine phosphatase (the positive regulator of Lck activity) is increased in T cells from patients with SLE [59].

6. Conclusions

In spite of many remaining unresolved issues and controversial aspects, the concept of lipid rafts has recently become remarkably influential and fruitful in many areas of cell biology, including molecular immunology. It has brought plausible explanations of many aspects of receptor signaling. It can be reasonably speculated that rational targeting of lipid rafts and their interactions with immunoreceptor signaling components may become interesting even for therapeutic purposes. However, as is also indicated from the present review, there are still many open questions to be resolved by further research. In my opinion, it will be very important not only to resolve the controversies about basic properties of the "conventional" detergent-resistant buoyant lipid rafts but also to find out whether and how many other—methodically less easily accessible—types of functionally relevant lipid-based microdomains exist in biological membranes.

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